2) Stangl R, Altendorf-Hofmann A, Charnley RM, *et al*: Factors influencing the natural history of colorectal liver metastases. *Lancet* 343: 1405-1410, 1994.
3) Yamashita K, Urakami A, Kubota H, *et al*: Results of hepatic arterial infusion chemotherapy with 5-FU and leucovorin for unresectable liver metastases from colorectal cancer. *Jpn J Cancer Chemother* 35(1): 71-76, 2008.
4) Wood CB, Gillis CR and Blumgart LH: A retrospective study of the natural history of patients with liver metastases from colorectal cancer. *Clin Oncol* 2: 285-288, 1976.
5) Registry of Hepatic Metastases: Resection of the liver for colorectal carcinoma metastases; a multiinstitutional study of indication for resection. *Surgery* 103: 278-288, 1988.
6) Scheele J, Stang R, Altendorf-Hofmann A, *et al*: Resection of colorectal liver metastases. *World J Surg* 19: 59-71, 1995.
7) Arai Y, Inaba Y, Takeuchi Y, *et al*: Intermittent hepatic arterial infusion of high-dose 5-FU on a weekly schedule for liver metastases from colorectal cancer. *Cancer Chemother Pharmacol* 40: 526-530, 1997.
8) Kerr DJ, McArdle CS, Ledermann J, *et al*: Intrahepatic arterial versus intravenous fluorouracil and folinic acid for colorectal cancer liver metastases. *Eur J Surg Oncol* 26: 468-473, 2000.
9) Alberts SR, Horvath WL, Stenfeld WC, *et al*: Oxaliplatin, fluorouracil, and leucovorin for patients with unresectable liver-only metastasis from colorectal cancer: a North Center Cancer Treatment Group Phase II study. *J Clin Oncol* 23(36): 9243-9249, 2005.
10) 渡辺伸治，田中邦哉，山口茂樹・他: 大腸癌肝転移の外科治療. 消化器外科 24: 293-301, 2001.
11) Ballantyne GH and Quin J: Surgical treatment of liver matastases in patients with colorectal cancer. *Cancer* 71: 4252-4266, 1993.
12) Lehnert T, Knaebel HP, Duck M, *et al*: Sequential hepatic and pulmonary resection for metastatic colorectal cancer. *Br J Surg* 86: 241-243, 1999.
13) Folprecht G, Grothey A, Alberts S, *et al*: Neoadjuvant treatment of unresectable colorectal liver metastases: correlation between tumour response and resection rates. *Ann Oncol* 16: 1311-1319, 2005.
14) Adam R, Lucidi V and Bismuth H, *et al*: Hepatic colorectal metastases: methods of improving resectability. *Surg Clin N Am* 84: 659-671, 2004.
15) Arai Y, Inaba Y, Matsuda K, *et al*: Weekly 5hour hepatic arterial infusion of high dose 5-FU for unresectable liver metastases from colorectal cancer in patients without extra-hepatic lesions. *Proc ASCO* 17: 285a, 1998.
16) 熊田 卓，荒井保明，伊藤和樹・他: 大腸癌肝転移に対する大量 5-FU 週 1 回 5 時間持続動注療法—多施設共同研究—. 日癌治療会誌 28: 1449, 1993.
17) Kemeny N, Neidzweiecki D and Hollis DR: Hepatic arterial infusion versus systemic therapy for hepatic metastases from colorectal cancer: a randomized trial of efficacy, quality of life, and molecular markers (CALGB 9481). *J Clin Oncol* 24: 1395-1403, 2006.
18) Ducreux M, Ychou M, Laplanche A, *et al*: Hepatic arterial oxaliplatin infusion plus intravenous chemotherapy in colorectal cancer with inoperable hepatic metastases: a trial of the gastrointestinal group of the Federation Nationale des Centres de Lutte Contre le Cancer. *J Clin Oncol* 23: 4883-4896, 2005.
19) Kemeny N, Jarnagin W, Paty P, *et al*: Phase I trial of systemic oxaliplatin combination chemotherapy with hepatic arterial infusion in patients with unresectable liver metastases from colorectal cancer. *J Clin Oncol* 23: 4888-4896, 2005.

World J Surg (2009) 33:312–317
DOI 10.1007/s00268-008-9831-2

Risk Factors of Surgical Site Infection After Hepatectomy for Liver Cancers

Shin Kobayashi · Naoto Gotohda · Toshio Nakagohri · Shinichiro Takahashi · Masaru Konishi · Taira Kinoshita

Published online: 21 November 2008

Abstract
Background Risk factors of surgical site infection (SSI) after hepatectomy under the guideline of Centers for Disease Control and Prevention (CDC) are not well examined.
Methods Hospital records of consecutive patients who underwent hepatectomy without biliary reconstruction for liver cancers were reviewed retrospectively. Prophylactic antibiotics were given to patients just before skin incision and every 3 hours during the operations. Clinicopathological factors were compared between patients who developed SSI and those without it.
Results There were 405 patients identified, and the incidence of SSI was 23 cases (5.8%). In multivariate analysis, intraoperative bowel injury, blood loss >2000 ml, and age older than 65 years were significant risk factors of SSI after hepatectomy.
Conclusions Prophylactic antibiotics were necessary only during the operation for most patients who underwent hepatectomy without biliary reconstruction. However, patients with intraoperative bowel injury, blood loss >2000 ml, and age older than 65 years are at risk to develop SSI and might need additional administration of prophylactic antibiotics after surgery.

Introduction

Use of antibiotics is one of the main techniques to prevent surgical site infection (SSI) after surgery. There has been tremendous accumulation of evidence during the last three decades with regard to the optimal methods of its administration [1]. The Centers for Disease Control and Prevention (CDC) recommended in its 1999 guideline to maintain therapeutic levels of prophylactic antibiotic during the operation and, at most, a few hours after closure of incisions [2]. However, it is well known that incidence of SSI is greatly influenced by patients' underlying general status and perioperative factors [3]. Disease and procedure-specific risks and use of prophylactic antibiotics are not well examined, except for colorectal surgery [4, 5], open heart surgery [6], cholecystectomy [7, 8], etc.

It is suggested that hepatectomy suppresses Kupffer cell and T-cell function significantly, which renders patients immunosuppressive [9]. Postoperative infection, including SSI, deteriorates hepatic failure in cases with limited hepatic functional reserve. There is a wide variety in operation time, blood loss, transfusion requirement, etc., depending on the extent of parenchymal resection. Underlying cirrhosis and hypoalbuminemia inhibits normal wound healing [10]. However, perioperative factors that should be considered a significant risk to develop SSI after hepatectomy have not been clear. The purpose of this study was to analyze the risk factors of SSI after hepatectomy with prophylactic antibiotics under CDC guideline and to clarify who might benefit from additional administration of prophylactic antibiotics after operation.

Materials and methods

Patients who underwent hepatectomy for liver cancers from November 2002 to December 2006 at National Cancer Center East Hospital, Kashiwa, Japan, were identified and reviewed retrospectively. Patients who

S. Kobayashi (✉) · N. Gotohda · T. Nakagohri · S. Takahashi · M. Konishi · T. Kinoshita
National Cancer Center East Hospital, 6-5-1, Chiba, Kashiwanoha, Kashiwa, Chiba 277-8577, Japan
e-mail: shkobaya@east.ncc.go.jp

underwent hepatectomy without biliary reconstruction regardless of diagnosis were included in the study. Patients who underwent cholecystectomy along with hepatectomy were included in the study, but those who underwent simultaneous procedures, such as colorectal resection or stoma closure, were excluded from the study.

The extent of hepatectomy was evaluated according to the disease progression, liver function, and general condition of patients [11]. Tumor progression and resectability was assessed by imaging studies, such as contrast enhanced computed tomography (CT) scans, magnetic resonance imaging (MRI), hepatic arterial angiography, ultrasound, and chest x-ray. Liver function was assessed by liver biochemistry test, Child-Pugh grade [12], and the indocyanine green retention rate at 15 minutes [13]. All patients were reviewed before surgery at weekly conferences by hepatic surgeons, medical oncologists, and interventional radiologists to discuss whether the planned procedures were appropriate. Hepatic resection was performed under intraoperative ultrasonographic guidance by the pean fracture method with or without inflow occlusion (Pringle's maneuver). Anatomic hepatectomy was performed whenever possible, whereas partial resection was performed in consideration of limited liver functional reserve or anatomic location of the tumor. During parenchymal resection, all blood vessels and bile ducts were ligated whenever possible with 2-0 or 3-0 braided silk or vessel clip. One or two closed drains were inserted at the end of operation in the right subphrenic space or wherever close to the resected liver parenchyma. Drains were removed when no rebleeding or bile leakage was observed on postoperative day (POD) 3 or 4.

SSI was defined as a condition in which purulent discharge was observed from any incision or space that was manipulated during an operation within 30 days after the operation with or without microbiological evidence as in the guideline issued by CDC [2], and it was identified retrospectively by reviewing clinical records of patients who underwent hepatectomy. Remote site infection was defined as a condition in which fever and leukocytosis were present with bacteria in sputum, urine, catheter-tip, blood, or other body fluid/space, or according to the physician's judgment regardless of microbiological evidence.

Patients were usually given two doses of cefazolin as prophylactic antibiotics. One gram of cefazolin was administered to patients within 30 minutes before skin incision and another dose 3 hours later. When the operation lasted more than 3 hours, additional doses were given every 3 hours thereafter during the operation. No antibiotics were given after incisions were closed if patients had already received two doses of cefazolin.

All data were compiled in a database for analysis (Microsoft Excel and SPSS 11.0 J for Windows). Differences between numerical variables were tested with Mann-Whitney U test and those between categorical variables were tested with χ^2 statistics. Multivariate analysis was performed with logistic regression test. $p < 0.05$ was deemed significant.

Results

During the period of study, 405 patients underwent hepatectomy without biliary reconstruction for primary or secondary liver cancers at National Cancer Center East Hospital, Kashiwa, Japan. Of these 405 patients, 23 patients (5.8%) developed SSI (incisional, 20; organ/space, 3). Incisional SSIs were treated by opening incisions and organ/space SSIs were treated by drainage under ultrasound guidance. The patient characteristics and demographic variables are listed in Table 1. No differences in these basic characteristics, except age, were observed between patients with SSI and those without it. Mean age of patients with SSI was 68.2 years and was statistically older than those without SSI. A cutoff value of aged 65 years had the highest statistical power ($p = 0.016$). Patients' ASA score, comorbidities, and underlying liver pathology were statistically similar between the two groups.

Culture results of infecting organisms included *Bacteroides faragilis* (n = 3), *Staphylococcus aureus* (n = 2), *Klebsiera oxytoca* (n = 1), *Serratia marcescens* (n = 1), *Escherichia coli* (n = 1), *Streptoccoccus anginosus* (n = 1), *Streptococcus constellatus* (n = 1), *Enterobacter cloacae* (n = 1), *Citrobacter braakii* (n = 1), *Citrobacter freundii* (n = 1), Corynebacterium species (n = 1), and Candida species (n = 1).

The perioperative variables are listed in Table 2. Operation time, red blood cell (RBC) transfusion requirement, RBC transfusion volume, and intraoperative bowel injury were statistically different between the two groups. Blood loss did not reach statistical significance, but cutoff value of 2000 ml had the significant power to predict SSI ($p = 0.003$). Multivariate analysis of those variables found that intraoperative bowel injury, blood loss >2000 ml, and age older than 65 years were the significant risk factors to develop SSI after hepatectomy without biliary reconstruction (Table 3). Rates of SSI increased dramatically with the number of risk factors present (Fig. 1). Patients with two or more risk factors were statistically more likely to develop SSI than those with none or only one risk factor.

During the same period, three patients died within 30 days from the operations. One patient died from pulmonary embolism on POD 3, another died from brain stroke on POD 3, and the other died from esophageal varix rupture on POD 9. Incidence of remote site infection was

Table 1 Patient characteristics and demographic variables for patients with SSI compared with those without it

	SSI (−) (N = 382)	SSI (+) (N = 23)	*P* value
Age (yr)[a]	63.7 ± 0.5	68.2 ± 2	*0.034*
≥65[b]	194 (50.9)	18 (78.3)	*0.016*
<65	188 (49.1)	5 (21.7)	
Gender[b]			0.809
Male	285 (74.6)	18 (78.3)	
Female	97 (25.4)	5 (21.7)	
Body mass index (kg/m^2)[a]	23.8 ± 0.6	23.6 ± 0.7	0.583
Diabetes mellitus[b]	75 (19.6)	1 (4.5)	0.095
ASA score[b]			0.488
1	111 (29.5)	7 (30.4)	
2	243 (64.6)	16 (69.6)	
3	22 (5.9)		
Diagnosis[b]			0.566
HCC	239 (62.6)	13 (56.5)	
Metastases	126 (33)	8 (34.8)	
Others	16 (4.5)	2 (8.7)	
Viral hepatitis serology[b]			0.858
HBV	51 (14)	3 (13)	
HCV	141 (38.7)	8 (34.8)	
HBV and HCV	7 (1.9)		
Liver parenchyma[b]			0.758
Chronic hepatitis	105 (29.6)	9 (39.1)	
Liver cirrhosis	93 (26.2)	5 (21.7)	
Child class[b]			0.634
A	355 (94.4)	21 (91.3)	
B	21 (5.6)	2 (8.7)	
ICG15R[a]	14.6 ± 0.4	15.5 ± 1.6	0.571

[a] Mann-Whitney *U* test

[b] χ^2 test

Data are numbers with percentages in parentheses or means ± standard error of the mean

ASA American society of anesthesiology, *HCC* hepatocellular carcinoma, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *ICG15R* indocyanin green 15 min retention rate

11 (2.5%) (pneumonia (n = 6), urinary tract infection (n = 1), catheter infection (n = 1), epididymitis (n = 1), unknown origin (n = 2)). Other morbidities included bile leak (n = 9), retractable ascites (n = 6), ileus (n = 4), transient renal insufficiency (n = 4), rebleeding (n = 3), pleural effusion (n = 3), skin rash (n = 2), poor oral intake (n = 2), delirium (n = 1), transient heart failure (n = 1), pulmonary embolism (n = 1), upper gastrointestinal bleeding (n = 1), wound dehiscence (n = 1). There were four reoperations for three rebleedings and one wound dehiscence.

Discussion

Our study clearly demonstrated the risk factors of SSI after hepatectomy with prophylactic antibiotics under the CDC guideline. Intraoperative bowel injury, blood loss >2000 ml, and age older than 65 years were the significant risk factors. Although both alimentary tract surgery and hepatobiliary surgery are classified as clean-contaminated [14], biliary tract without calculus is normally sterile contrary to the alimentary tract, which has high bacterial densities [15, 16]. Intraoperative bowel injury is suspected to contaminate surgical field of hepatectomy without biliary reconstruction and to increase the risk of SSI. Blood loss reduces the concentration of antibiotics and is found to be a risk factor of SSI [17, 18]; 1500 ml to 2000 ml of blood loss is the suggested threshold to administer additional doses of cefazolin to maintain a concentration higher than the minimum inhibitory concentration for the common infecting organisms [19, 20]. Our threshold of 2000 ml of blood loss is compatible with previous findings. Elderly patients also are reported to be susceptible to SSI [18, 21]. Because aging involves complex physiologic changes, it is difficult to clarify a definitive mechanism of the vulnerability of elderly patients. Reduction in immune function is one suggested mechanism [10].

Rates of SSI increased dramatically with the number of the three risk factors present (Fig. 1). According to the National Nosocomical Infections Surveillance (NNIS) report, rates of SSI after hepatopancreaticobiliary complex

Table 2 Perioperative variables for patients with SSI compared with those without it

	SSI (−) (N = 382)	SSI (+) (N = 23)	*P* value
Operation time (min)[a]	210 ± 19	269 ± 23	*0.021*
≥300[b]	68 (17.8)	9 (39.1)	*0.017*
<300	313 (82.2)	14 (60.9)	
Pringle time (min)[a]	63.3 ± 2.1	75.9 ± 9.7	0.259
None[b]	26 (7.3)	0 (0)	0.23
>0	331 (92.7)	20 (100)	
Repeat resection[b]	110 (28.8)	4 (17.4)	0.338
Blood loss (ml)[a]	1070 ± 69	1928 ± 470	0.068
≥2000[b]	50 (13.2)	9 (39.1)	*0.003*
<2000	332 (86.8)	14 (60.9)	
RBC transfusion (ml)[a]	177 ± 29	537 ± 192	*0.003*
None[b]	297 (78.2)	12 (52.2)	*0.009*
>0	83 (21.8)	11 (47.8)	
Intraoperative bowel injury[b]	3 (0.8)	4 (17.4)	*<0.001*
Bile leak[b]	7 (1.8)	2 (22.2)	0.087
Resected segments (Couinaud)[b]			0.96
<2	285 (74.8)	16 (69.6)	
2–3	42 (11)	3 (13)	
≥4	54 (14.2)	4 (17.4)	
Resected weight (g)[a]	221 ± 19	269 ± 77	0.281
Largest tumor size (cm)[a]	3.8 ± 0.2	3.7 ± 0.4	0.253
NNIS index[b]			0.184
0	293 (76.9)	14 (60.9)	
1	86 (22.6)	9 (39.1)	
2	2 (0.5)		
Postoperative length of stay[a]	10.2 ± 0.2	23.7 ± 5.7	*<0.001*

[a] Mann-Whitney *U* test

[b] χ^2 test

Data are numbers with percentages in parentheses or means ± standard error of the mean

RBC red blood cell, *NNIS* national nosocomical infection surveillance

Table 3 Multivariate analysis of SSI risk factors

	P value	Odds ratio (95% confidence intervals)
Age ≥65 yr	*0.027*	3.4 (1.15–10.05)
Blood loss ≥2000 ml	*0.004*	4.4 (1.63–11.91)
Intraoperative bowel injury	*<0.001*	20.08 (4–100.8)
RBC transfusion	0.62	1.51 (0.31–7.42)
Operation time >300 min	0.67	1.35 (0.34–5.32)

SSI risk factors identified by univariate analysis were compared by multivariate analysis (logistic regression test)

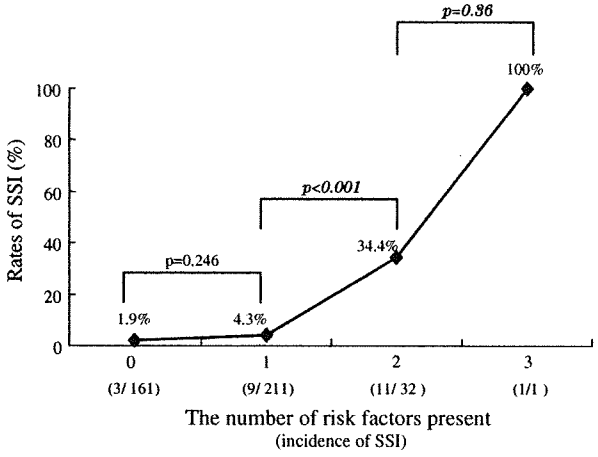


Fig. 1 Rates of SSI increased with the number of risk factors present. Rates of SSI were not statistically different between patients with one risk factor and those without any factors. However, patients with two or more risk factors developed SSI at a significantly higher rate than those with none or only one risk factor

surgery range from 3.24–7.04% [22]. Other reported rates of SSI after hepatectomy range from 4.6–25.2% [23, 24]. Compared with those previously reported rates, the rates of SSI for patients with none or only one risk factor, 1.9% and 4.3% respectively, are considered allowable. Prophylactic antibiotics for hepatectomy without biliary reconstruction are necessary only during operations for patients with none or only one risk factor. However, patients with two or more risk factors developed SSI at statistically higher rates. Fujita et al. [4] reported that two additional doses of postoperative antibiotics reduced the incidence of incisional SSI from 14.2% to 4.3% compared with single-dose preoperative administration in elective colorectal surgery

[4]. Additional administration of postoperative antibiotics maintains therapeutic levels for longer hours and reduces the incidence of SSI more effectively for patients at higher risk. Although there have been no published data concerning the effectiveness of postoperative administration of antibiotics in hepatectomy, Fig. 1 illustrates that patients with two or more risk factors may receive some additional doses of postoperative antibiotics as in colorectal surgery. Appropriate doses of additional antibiotics are matters to be discussed.

There were five infecting organisms that were resistant to cefazolin: *Bacteroides fragilis*, *Enterobacter, cloacae*, *Serratia marcescens*, Corynebacterium species, and Citrobacter species. Because some patients lack microbiologic data, a definitive conclusion about the optimum choice of prophylactic antibiotics was not possible. However, it is evident that cefazolin alone was effective for most patients who underwent hepatectomy without biliary reconstruction. Two of the seven patients with intraoperative bowel injury developed SSI with *Bacteroides fragilis*. Because likely pathogens in alimentary tract surgery are gram-negative bacilli and anaerobes [2], postoperative antibiotics with anaerobic coverage might be more effective for patients with intraoperative bowel injury.

Postoperative infections, especially organ/space SSI, sometimes deteriorate hepatic function and may cause mortalities. We experienced 23 SSIs and 11 remote site infections, but none of the patients died from those infections. We speculate that our strict evaluation of extent of hepatectomy using CT volumetry and liver function test precluded some excessive hepatic resection and saved postoperative hepatic function. Postoperative infection is more likely to occur in patients with hepatic dysfunction [25]. Our relatively low rate of major hepatectomy in consideration of hepatic functional reserve might be related to the fewer incidence of SSI.

RBC transfusion requirement and operation time were significant risk factors of SSI in univariate analysis, but not in multivariate analysis. Transfusion has immunosuppressive effects on postoperative patients via reductions in natural killer cell number and cytotoxic T-cell function [26, 27] and is reported to be a risk factor of SSI in colorectal surgery [28, 29]. However, controversy exists concerning the causal relationship between transfusion and SSI [30], and a recent meta-analysis denies the association between transfusion and postoperative infection [31]. Our result is consistent with the meta-analysis. Operation time is another reported risk factor of SSI [18]. Cefazolin exhibits time-dependent decrease in serum and tissue concentration, and additional administrations are recommended every 3 or 4 hours during operation to maintain therapeutic levels of cefazolin [2]. Because all of our patients received a second dose of cefazolin at 3 hours from incision, serum and tissue concentration of cefazolin was expected to exceed therapeutic levels during the whole time of operations for most patients. Influence of operation time on the incidence of SSI was suspected to be minimized with additional dose of cefazolin at 3 hours from incision.

Abdominal drainage after elective hepatectomy is controversial. Some randomized, controlled trials (RCTs) reported increased incidence of SSI and other morbidities associated with abdominal drainage and denied the routine placement of drainage catheters [32, 33]. However, the routine drainage group in those RCTs had drainage catheters placed for at least 5 to 9 days, which was unnecessarily long. We almost routinely placed drainage catheters but removed them on POD 3/4 or earlier if postoperative bleeding and bile leakage were denied. Early removal of prophylactic drains prevents intra-abdominal infections [34]. We do not consider that abdominal drainage causes more infections if drains are removed on POD 3/4 or earlier.

Our study has several limitations. First, SSI was detected indirectly by retrospectively reviewing patient records and laboratory data. It has been suggested to be a less accurate method than prospective direct observation of surgical sites [2]. Some SSI might be possibly undetected because of inappropriate patient records. However, indirect case-finding by reviewing daily records and laboratory data is the most widespread method of surveillance in the medical literature. Its reported sensitivity is as high as 83.8–92.3% compared with prospective direct finding of SSI [35]. Since then, we do not consider that our surveillance method precludes the importance of our findings. Second, it is a single-center study. Our department is one of the highest volume centers in Japan and performs 250 hepatopancreaticobiliary cancer surgeries in a year. Also, we do not perform operations on patients with end-stage renal disease on dialysis due to inadequacies of dialysis facilities. Our relatively low rate of SSI incidence may be attributable to the high volume of cases and to the patient selection.

Conclusions

Our study demonstrated that prophylactic antibiotics were necessary only during operations and, at most, a few hours after closure of incisions in most of the patients who underwent hepatectomy without biliary reconstruction. However, patients with intraoperative bowel injury, blood loss >2000 ml, and age older than 65 years were at risk for developing SSI. Patients with two or more risk factors may receive additional doses of postoperative antibiotics to prevent SSI more effectively.

References

1. Nichols RL (1996) Surgical infections: prevention and treatment-1965 to 1995. Am J Surg 172:68–74
2. Mangram AJ, Horan TC, Pearson ML et al (1999) Guideline for prevention of surgical site infection, 1999. Infect Control Hosp Epidemiol 20:247–278
3. Culver DH, Horan TC, Gaynes RP et al (1991) Surgical wound infection rates by wound class, operative procedure, and patient risk index. Am J Med 91(3B):152S–157S
4. Fujita S, Saito N, Yamada T et al (2007) Randomized, multicenter trial of antibiotic prophylaxis in elective colorectal surgery. Arch Surg 142:657–661
5. Tang R, Chen HH, Wang YL et al (2001) Risk factors for surgical site infection after elective resection of the colon and rectum: a single-center prospective study of 2, 809 consecutive patients. Ann Surg 234:181–189
6. Harbarth S, Samore MH, Lichtenberg D et al (2000) Prolonged antibiotic prophylaxis after cardiovascular surgery and its effect on surgical site infections and antimicrobial resistance. Circulation 101:2916–2921
7. Dobay KJ, Freier DT, Albear P (1999) The absent role of prophylactic antibiotics in low-risk patients undergoing laparoscopic cholecystectomy. Am Surg 65:226–228
8. Higgins A, London J, Charland S et al (1999) Prophylactic antibiotics for elective laparoscopic cholecystectomy: are they necessary? Arch Surg 134:611–613
9. Karpoff HM, Tung C, Ng B et al (1996) Interferon gamma protects against hepatic tumor growth in rats by increasing Kupffer cell tumoricidal activity. Hepatology 24:374–379
10. Silverstein JH (2002) Physiologic changes associated with aging. In: O'Leary JP (ed) The physiologic basis of surgery, 2nd edn. Lippincott Williams & Wilkins, Philadelphia, pp 705–711
11. Makuuchi M, Kosuge T, Takayama T et al (1993) Surgery for small liver cancers. Semin Surg Oncol 9:298–304
12. Pugh RN, Murray-Lyon IM, Dawson JL et al (1973) Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60:646–649
13. Lau H, Man K, Fan ST et al (1997) Evaluation of preoperative hepatic function in patients with hepatocellular carcinoma undergoing hepatectomy. Br J Surg 84:1255–1259
14. Talbot TR, Kaiser AB (2005) Postoperative infections and antimicrobial prophylaxis. In: Mandell GL (ed) Principles and practice of infectious diseases, 6th edn. Elsevier, Philadelphia
15. Fry DE (2002) Surgical infection. In: O'Leary JP (ed) The physiologic basis of surgery, 3rd edn. Lippincott Williams & Wilkins, Philadelphia, pp 212–258
16. Ahrendt SA, Pitt HA (2001) Biliary tract. In: Townsend CMJ (ed) Textbook of surgery. The biological basis of modern surgical practice, 16th edn. Saunders, Philadelphia, pp 1076–1111
17. Griffiths J, Demianczuk N, Cordoviz M et al (2005) Surgical site infection following elective caesarian section: a case-control study of postdischarge surveillance. J Obstet Gynaecol Can 27:340–344
18. Yamamoto S, Kanamaru S, Kunishima Y et al (2005) Perioperative antimicrobial prophylaxis in urology: a multi-center prospective study. J Chemother 17:189–197
19. Swoboda SM, Merz C, Kostuik J et al (1996) Does intraoperative blood loss affect antibiotic serum and tissue concentrations? Arch Surg 131:1165–1171
20. Meter JJ, Polly DWJ, Brueckner RP et al (1996) Effect of intraoperative blood loss on the serum level of cefazolin in patients managed with total hip arthroplasty. A prospective, controlled study. J Bone Joint Surg Am 78:1201–1205
21. Yoshida J, Shinohara M, Ishikawa M et al (2006) Surgical site infection in general and thoracic surgery: surveillance of 2663 cases in a Japanese Teaching Hospital. Surg Today 36:114–118
22. Gaynes RP, Culver DH, Horan TC et al (2001) Surgical site infection (SSI) rates in the United States, 1992–1998: The National Nosocomial Infections Surveillance System Basic SSI Risk Index. Clin Infect Dis 33(2):S69–S77
23. Togo S, Matsuo K, Tanaka K et al (2007) Perioperative infection control and its effectiveness in hepatectomy patients. J Gastroenterol Hepatol 22:1942–1948
24. Wu CC, Yeh DC, Lin MC et al (1998) Prospective randomized trial of systemic antibiotics in patients undergoing liver resection. Br J Surg 85:489–493
25. Schindl MJ, Redhead DN, Fearon KC et al (2005) The value of residual liver volume as a predictor of hepatic dysfunction and infection after major liver resection. Gut 54:289–296
26. Gascon P, Zoumbos NC, Young NS (1984) Immunological abnormalities in patients receiving multiple blood transfusions. Ann Intern Med 100:173–177
27. Kaplan J, Samaik S, Gitlin J et al (1984) Diminished helper/suppressor lymphocyte ratios and natural killer activity in recipients of repeated blood transfusions. Blood 64:308–310
28. Vamvakas EC, Carven JH, Hibberd PL (1996) Blood transfusion and infection after colorectal cancer surgery. Transfusion 36:1000–1008
29. Mynster T, Christensen IF, Moesgaard F et al (2000) Effects of the combination of blood transfusion and postoperative infectious complications on prognosis after surgery for colorectal cancer. Br J Surg 87:1553–1562
30. Vamvakas EC, Carven JH (1998) Transfusion of white-cell-containing allogeneic blood components and postoperative wound infection: effect of confounding factors. Transfus Med 8:29–36
31. Vamvakas EC (2007) White-blood-cell-containing allogeneic blood transfusion and postoperative infection or mortality: an updated meta-analysis. Vox Sang 92:224–232
32. Fong Y, Brennan M, Brown K et al (1996) Drainage is unnecessary after elective liver resection. Am J Surg 171:158–162
33. Liu CL, Fan ST, Lo CM et al (2004) Abdominal drainage after hepatic resection is contraindicated in patients with chronic liver disease. Ann Surg 239:194–201
34. Kawai M, Tani M, Terasawa H et al (2006) Early removal of prophylactic drains reduces the risk of intra-abdominal infections in patients with pancreatic head resection. Ann Surg 244:1–7
35. Cardo DM, Falk PS, Mayhall CG (1993) Validation of surgical wound surveillance. Infect Control Hop Epidemiol 14:211–215

Surg Endosc (2009) 23:906–908
DOI 10.1007/s00464-008-0257-6

Safe and feasible inflow occlusion in laparoscopic liver resection

Akihiro Cho · Hiroshi Yamamoto · Matsuo Nagata · Nobuhiro Takiguchi · Hideaki Shimada · Osamu Kainuma · Hiroaki Souda · Hisashi Gunji · Akinari Miyazaki · Atsushi Ikeda · Ikuko Matsumoto

Received: 28 March 2008 / Accepted: 15 November 2008 / Published online: 31 December 2008

Abstract
Background A major challenge in laparoscopic liver resection to avoid massive hemorrhage from the transection plane.
Methods This study investigated 32 consecutive patients who underwent laparoscopic or laparoscopically assisted hepatic resection and had the hepatoduodenal ligament encircled by vessel tape using an Endo Retract Maxi as a tourniquet for complete interruption of blood inflow to the liver.
Results Laparoscopic encircling of the hepatoduodenal ligament was performed in a few minutes without any complications for any of the 32 patients.
Conclusions Laparoscopic Pringle's maneuver using an Endo Retract Maxi can be performed easily for all patients undergoing laparoscopic liver resection.

Keywords Hepatectomy · Laparoscopy · Pringle's maneuver

Minimally invasive surgery has become widely accepted as a superior alternative to conventional open surgery in many gastrointestinal fields. Moreover, recent rapid developments in technological innovations, improved surgical techniques, and accumulation of extensive experience by surgeons have improved the feasibility and safety of laparoscopic liver surgery [1–4]. However, laparoscopy for liver resection remains a highly specialized field because laparoscopic liver surgery presents severe technical difficulties, such as control of hemorrhage from the transection plane.

Laparoscopy for major liver resection remains uncommon, partly because of the potential for massive hemorrhage. In particular, hepatocellular carcinoma usually occurs from a cirrhotic liver, which often causes bleeding problems. In addition, massive intraoperative blood loss is a good predictor of postoperative morbidity and mortality for patients who undergo liver resection for hepatic malignancies [5, 6]. Intraoperative inflow occlusion of the liver has thus been recommended to reduce blood loss during liver resection [7, 8].

Although various techniques of hepatic vascular control have been presented, Pringle's maneuver, the oldest and simplest, still is favored by many surgeons. However, laparoscopic encircling of the hepatoduodenal ligament can prove difficult because the field of view is narrow and the surgeon's blind spot may lead to unexpected bleeding or injury under laparoscopy.

In this report we describe a new technique whereby any surgeon with minimal or no laparoscopic experience can easily and safely perform Pringle's maneuver during laparoscopic liver resection.

Surgical procedure

The patient is placed in supine position with legs apart. The surgeon stands between the legs. A 12-mm trocar is placed 1 cm below the umbilicus, through which carbon dioxide gas is delivered. Pneumoperitoneum is controlled electronically to a pressure of 10 mmHg. Additional working ports are placed to optimize manipulation and mobilization

A. Cho (✉) · H. Yamamoto · M. Nagata · N. Takiguchi · H. Shimada · O. Kainuma · H. Souda · H. Gunji · A. Miyazaki · A. Ikeda · I. Matsumoto
Division of Gastroenterological Surgery, Chiba Cancer Center Hospital, 666-2 Nitonachou, Chuouku, Chiba 260-8717, Japan
e-mail: acho@chiba-cc.jp

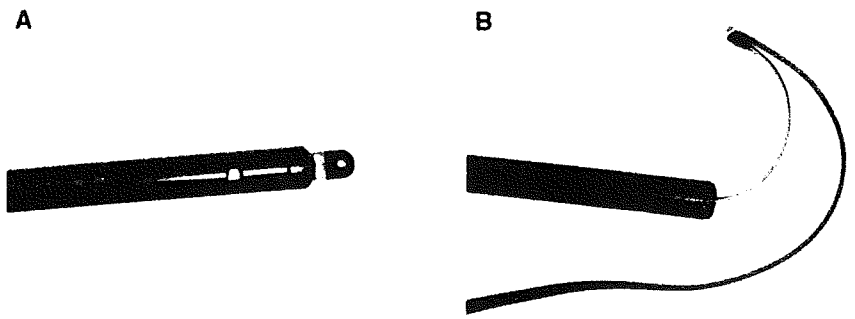


Fig. 1 (**A**) Endo Retract Maxi in closed position. (**B**) Endo Retract Maxi in activated position. Vessel tape has been preliminarily fixed to the tip of the metallic arch

of the liver, as described previously [4]. Laparoscopic encircling of the hepatoduodenal ligament usually is performed using an Endo Retract Maxi (United Surgical, a division of Tyco Healthcare Group LP, Norwalk, CT, USA) to which silicon tape (Vesseloops; Argon Medical Devices, TX, USA) is fixed preliminarily with suture securing vessel tape to the tip (Fig. 1). The lesser omentum is sectioned. Because a space exists between the hepatoduodenal ligament and the inferior vena cava, it is not necessary to divide any layers other than the lesser omentum.

The Endo Retract Maxi in closed position is inserted via a 12-mm trocar into the upper median or the left lumbar quadrant and advanced from an opening through the lesser omentum to Winslow's foramen. The metallic arch with vessel tape then is meticulously extended behind the hepatoduodenal ligament, allowing visualization of the tip with vessel tape at the right side of the hepatoduodenal ligament (Fig. 2).

Although the Endo Retract Maxi is blindly deployed between the hepatoduodenal ligament and the inferior vena cava, the tip can be delivered safely into the right side of the hepatoduodenal ligament because the blade is blunt. The vessel tape is grasped with laparoscopic forceps, divided with laparoscopic scissors, and separated from the Endo Retract Maxi. The Endo Retract Maxi then is pulled from the lesser omentum. Both ends of the vessel tape are pulled from the abdominal cavity to the upper median trocar and used as a tourniquet for complete interruption of blood inflow to the liver (Fig. 3). If hemihepatic inflow occlusion is necessary, the left or right Glissonean pedicles are encircled using an Endo Retract Maxi at the hepatic hilum, as described previously [4]. The Nelaton catheter (Terumo, Tokyo, Japan) through which both ends of the vessel tape are passed is inserted and pushed via the upper median 12-mm trocar and secured using the forceps to tighten the hepatoduodenal ligament down around the pedicle (Fig. 3).

Fig. 2 The metallic arch of the Endo Retract Maxi is moved behind the hepatoduodenal ligament (HDL) so the tip with vessel tape is visualized at the right side of the HDL

A total of 32 consecutive patients who underwent laparoscopic or assisted hepatic resection at Chiba Cancer Center Hospital had the hepatoduodenal ligament encircled by vessel tape using an Endo Retract Maxi as a tourniquet for complete interruption of blood inflow to the liver if necessary. In all 32 patients, laparoscopic encircling of the hepatoduodenal ligament using an Endo Retract Maxi was easily and rapidly performed without any complications, even by surgeons with minimal or no laparoscopic experience.

Discussion

Recent technological developments and improved endoscopic procedures have further spread the application of laparoscopic liver resection. A major challenge with this procedure is to avoid massive hemorrhage from the transection plane. Pringle's maneuver has been widely used to reduce intraoperative blood loss because this technique is easily performed in conventional open surgery. However, this maneuver is not so easily performed under laparoscopic circumstances because the curve of the laparoscopic forceps usually is too obtuse to encircle the hepatoduodenal ligament. In addition, the tip of the laparoscopic forceps is sharp and hard, and thus has the potential to injure organs under blind manipulation. Although a biliary scope is very

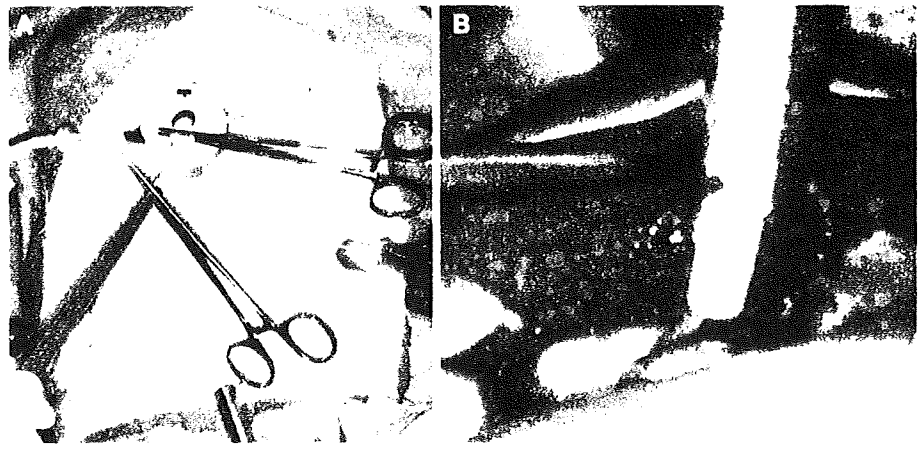


Fig. 3 Both ends of the vessel tape are pulled from the abdominal cavity to the upper median trocar (**A**) and used as a tourniquet for complete interruption of blood inflow to the liver (**B**)

useful for encircling the hepatoduodenal ligament [9], preparing and manipulating a biliary scope may be somewhat problematic and time consuming.

For the current procedure, no special instrument except an Endo Retract Maxi is necessary. Laparoscopic encircling of the hepatoduodenal ligament using an Endo Retract Maxi was performed in a few minutes without any of the 32 patients undergoing this approach experiencing any complications. Although our experience is limited, we believe that laparoscopic encircling of the hepatoduodenal ligament using an Endo Retract Maxi is easily performed for all patients undergoing laparoscopic liver resection.

References

1. Kaneko H, Takagi S, Shiba T (1996) Laparoscopic partial hepatectomy and left lateral segmentectomy: technique and results of a clinical series. Surgery 120:468–475
2. Cherqui D, Husson E, Hammoud R et al (2000) Laparoscopic liver resections: a feasibility study in 30 patients. Ann Surg 232:753–762
3. Shimada M, Hashizume M, Maehara S et al (2001) Laparoscopic hepatectomy for hepatocellular carcinoma. Surg Endosc 15:541–544
4. Cho A, Asano T, Yamamoto H et al (2007) Laparoscopy-assisted hepatic lobectomy using hilar Glissonean pedicle transection. Surg Endosc 21:1466–1468
5. Nagasue N, Kohno H, Chang YC et al (1993) Liver resection for hepatocellular carcinoma: results of 229 consecutive patients during 11 years. Ann Surg 217:375–384
6. Hanazaki K, Kajikawa S, Shimozawa N et al (2000) Survival and recurrence after hepatic resection of 386 consecutive patients with hepatocellular carcinoma. J Am Coll Surg 191:81–88
7. Dixon E, Vollmer CM Jr, Bathe OF, Sutherland F (2005) Vascular occlusion to decrease blood loss during hepatic resection. Am J Surg 190:75–86
8. Smyrniotis V, Farantos C, Kostopanagiotou G, Arkadopoulos N (2005) Vascular control during hepatectomy: review of methods and results. World J Surg 29:1384–1396
9. Maehara S, Adachi E, Shimada M et al (2007) Clinical usefulness of biliary scope for Pringle's maneuver in laparoscopic hepatectomy. J Am Coll Surg 205:816–818

Messenger RNA expression of COX-2 and angiogenic factors in primary colorectal cancer and corresponding liver metastasis

HIROTOSHI KOBAYASHI[1,2], KENICHI SUGIHARA[1], HIROYUKI UETAKE[1], TETSURO HIGUCHI[1], MASAMICHI YASUNO[1], MASAYUKI ENOMOTO[1], SATORU IIDA[1], HEINZ-JOSEF LENZ[3], KATHLEEN D. DANENBERG[4] and PETER V. DANENBERG[2]

[1]Department of Surgical Oncology, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan; Departments of [2]Biochemistry and Molecular Biology and [3]Medical Oncology, University of Southern California/Norris Comprehensive Cancer Center; [4]Response Genetics, Inc., Los Angeles, CA, USA

Received December 5, 2008; Accepted January 29, 2009

DOI: 10.3892/ijo_00000243

Abstract. Several new drugs that are targeted towards various angiogenic factors have shown considerable potential for controlling tumor proliferation and metastases. Expression levels of the targeted genes in primary tumors and metastases should be understood to maximize the use of such drugs. The present study aimed to clarify associations between mRNA levels of cyclooxygenase 2 (COX-2) and angiogenic factors [vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8)] in primary colorectal cancer and in corresponding liver metastasis. We also compared these gene expressions of primary colorectal cancer between patients with and without liver metastasis. In 31 pairs of formalin-fixed and paraffin-embedded primary and metastatic liver tumors as well as 27 specimens of consecutive stage II patients without recurrence, mRNA was quantified by real-time reverse transcription-polymerase chain reaction following the laser capture microdissection. We found a significantly positive correlation in *IL-8* between primary tumors and matched liver metastases ($p=0.034$, $r_s=0.39$) and in VEGF ($p=0.0083$, $r_s=0.48$), but not in *COX-2*, which was associated with both *VEGF* ($p=0.044$, $r_s=0.37$) and *IL-8* ($p=0.0004$, $r_s=0.64$) in primary colorectal cancers. Multiple regression analysis revealed that *COX-2* was independently associated with *IL-8* ($p<0.0001$). There were no differences in mRNA levels between patients with and without liver metastasis. The mRNA levels of VEGF and IL-8 in liver metastasis can be predicted from those in primary colorectal cancer. COX-2 might exert angiogenic activity more through the IL-8, than the VEGF pathway. These angiogenic factors were sufficiently up-regulated before hematogenous metastasis. These preliminary data merit further validation studies.

Correspondence to: Dr Hirotoshi Kobayashi, Department of Surgical Oncology, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo, Japan
E-mail: h-kobayashi.srg2@tmd.ac.jp

Key words: colon cancer, liver metastasis, cyclooxygenase-2, vascular endothelial growth factor, interleukin-8, reverse transcription-polymerase chain reaction, messenger RNA

Introduction

Colorectal cancer is a worldwide leading cause of cancer death (1,2). The most promising treatment for patients with colorectal cancer is curative resection, but this is sometimes impossible. Some patients with colorectal cancer constantly relapse despite curative resection (3). Molecular targeting therapy has recently been developed for advanced colorectal cancer. Various drugs targeting anti-angiogenesis have improved the survival of patients with metastatic colorectal cancer (4), because angiogenesis is essential for tumor growth (5).

Interleukin-8 (IL-8) is a pro-inflammatory chemotactic cytokine that stimulates the migration of cells including neutrophils, monocytes, lymphocytes, and fibroblasts (6-9). The angiogenic activity of IL-8 produced by monocytes and macrophages was originally demonstrated in 1992 (10). Several investigators have reported that IL-8 is also secreted by some human colorectal cancer cells. Studies have shown that the range of IL-8 expression is 45-74% in colorectal cancer (11,12). However, details of IL-8 messenger RNA (mRNA) expression in colorectal cancer and corresponding liver metastasis remain unclear.

Senger *et al* originally identified the vascular endothelial growth factor (VEGF), which promotes angiogenesis, in 1983 (13). Bevacizumab is a monoclonal antibody to VEGF that has improved the survival of patients with metastatic colorectal cancer when combined with other chemotherapies (4).

Cyclooxygenase (COX) is a key enzyme that is involved in the conversion of arachidonic acid to prostaglandins. The COX-2 isoform is expressed in most organs, but can be up-regulated by various factors including cytokines, growth factors and tumor promoters (14,15). Recent studies have demonstrated that COX-2 inhibitors exert angiogenic effects

in colorectal cancer (16,17). However, the association of mRNA between COX-2 and angiogenic factors in colorectal cancer remain unclear.

Several novel drugs that are targeted towards various angiogenic factors have shown considerable potential for controlling tumor proliferation and metastasis. To maximize the effects of such drugs, correlations between expression levels of targeted genes in primary tumors and metastases should be determined. The present study examines associations between the mRNA levels of COX-2 and angiogenic factors such as VEGF and IL-8 in primary colorectal cancer and in corresponding liver metastasis. We also evaluated the association between COX-2 and angiogenic factors.

Patients and methods

Patients. We enrolled 31 patients who had undergone surgical resection for both primary colorectal cancer and liver metastasis between April 1997 and June 2005 at Tokyo Medical and Dental University Hospital. Of these, 18 and 13 had metachronous and synchronous liver metastases, respectively. The median time from primary resection to hepatectomy was 20 months. We compared mRNA expression between primary colorectal cancer and corresponding liver metastases. We also enrolled 27 patients who had undergone curative resection for stage II colorectal cancer between January 1998 and August 2001 and who had not relapsed during a median follow-up of 4.8±1.1 years. We then compared mRNA expression between the 31 patients with liver metastasis (Group 1) and the 27 stage II patients without relapse (Group 2). Patients with ulcerative colitis, Crohn's disease, or familial adenomatous polyposis were excluded from this study, which was approved by the institutional review board of Tokyo Medical and Dental University, and all patients provided written, informed consent to participate. None of the patients had undergone prior radiotherapy or chemotherapy. Table I summarizes their clinical and histopathological data.

Laser capture microdissection. Formalin-fixed paraffin-embedded tumor tissue blocks were cut into 10-μm-thick slices, stained with nuclear fast red (American MasterTech Scientific, Lodi, CA) and then laser capture microdissection (P.A.L.M. Microlaser Technologies AG, Munich, Germany) was applied. This technique allows only tumor cells to be examined with stromal tissues removed.

RNA isolation and cDNA synthesis. After laser capture microdissection, RNA was isolated according to the proprietary procedure of Response Genetics (US patent no. 6,248,535) and then cDNA was prepared from each sample as described (18).

Quantitative reverse transcription polymerase chain reaction (RT-PCR). Genes of interest and an internal reference gene (ß-actin) were quantified using fluorescence-based real-time TaqMan detection (ABI PRISM 7900 Sequence Detection System; Applied Biosystems, Foster City, CA) as described (19) and the specific mRNA amplification primers and probes were listed in Table II. The PCR mixture comprised 1,200 nmol/l of each primer, 200 nmol/l probe, 0.4 U of AmpliTaq Gold Polymerase, 200 nmol/l each of dATP,

Table I. Clinicopathological characteristics.

	Group 1 (n=31 with liver metastasis)	Group 2 (27 stage II without relapse)	P-value
Age (years)	61±9	69±11	0.0035
Gender			
Male	24	15	NS
Female	7	12	
Primary site			
Cecum	1	0	NS
Ascending colon	3	4	
Transverse colon	3	5	
Descending colon	2	1	
Sigmoid colon	9	9	
Rectosigmoid	9	4	
Rectum	4	4	
Pathology (differentiation)			
Well	12	11	NS
Moderate	17	15	
Poor	1	1	
Mucinous type	1	0	
Depth of tumor			
T1	0	0	NS
T2	2	0	
T3	21	25	
T4	8	2	
Lymph node metastasis			
N0	9	27	<0.0001
N1	13	0	
N2	9	0	
Lymphatic invasion			
Absent	4	8	0.013
Minimal	13	17	
Moderate	12	2	
Severe	2	0	
Venous invasion			
Absent	0	5	NS
Minimal	13	11	
Moderate	11	8	
Severe	7	3	

Table II. Primer and probe sequences of analyzed genes.

	Sequences
COX-2	
Forward primer	5'-GCTCAAACATGATGTTTGCATTC-3'
Reverse primer	5'-GCTGGCCCTCGCTTATGA-3'
Probe	5'-(FAM)TGCCCAGCACTTCACGCATCAGTT(TAMRA)-3'
IL-8	
Forward primer	5'-CAGCTCTGTGTGAAGGTGCAGTT-3'
Reverse primer	5'-GGGTGGAAAGGTTTGGAGTATGTC-3'
Probe	5'-(FAM)TGCACTGACATCTAAGTTCTTTAGCACTCCTTGGC(TAMRA)-3'
VEGF	
Forward primer	5'-AGTGGTCCCAGGCTGCAC-3'
Reverse primer	5'-TCCATGAACTTCACCACTTCGT-3'
Probe	5'-(FAM)ATGGCAGAAGGAGGAGGGCAGAATCA(TAMRA)-3'
ß-actin	
Forward primer	5'-TGAGCGCGGCTACAGCTT-3'
Reverse primer	5'-TCCTTAATGTCACGGACGATTT-3'
Probe	5'-(FAM)ACCACCACGGCCGAGCGG(TAMRA)-3'

dCTP, dGTP, dTTP, 3.5 mmol/l $MgCl_2$, and 1X TaqMan buffer A containing a reference dye in a final volume of 20 μl (all reagents were supplied by Perkin-Elmer Applied Biosystems). The cycling conditions comprised 50°C for 2 min and 95°C for 10 min followed by 46 cycles at 95°C for 15 sec and 60°C for 1 min. Gene expression is expressed as ratios (relative mRNA levels) between genes of interest and the internal reference ß-actin gene. All samples were amplified in triplicate.

Statistical analysis. Data were statistically analyzed using the StatView statistical package (StatView 5.0, Abacus Concepts, Inc., Berkeley, CA, USA). All data are expressed as median ± standard deviation. We compared the mRNA levels of genes of interest between primary colorectal cancer and corresponding liver metastasis using the Wilcoxon's signed-rank test. Spearman's rank correlation analysis determined correlations between mRNA levels of primary tumor and liver metastases and associations between mRNA levels of COX-2 and angiogenic factors. Associations between clinicopathological features and mRNA expression were assessed by the Mann-Whitney U test with two variables and by the Kruskal-Wallis test with three or more variables. Statistical significance was established at $p<0.05$ for all values.

Results

Table I shows the clinicopathological features of the patients. Those with stage II colorectal cancer whose cancer did not recur were older than those with liver metastasis ($p=0.0035$). The extent of lymph node metastasis and lymphatic invasion significantly differed between the two groups ($p<0.0001$ and $p=0.013$, respectively). The mRNA levels of each gene did not differ between patients with primary colorectal cancer accompanied by synchronous or metachronous liver metastasis (Fig. 1A). The mRNA levels of primary tumors also did not significantly differ between patients with solitary or multiple liver metastases (Fig. 1B).

Correlation in mRNA expression between primary colorectal cancer and corresponding liver metastasis. The expression of COX-2 mRNA did not significantly differ between primary colorectal cancer and corresponding liver metastasis from 31 patients (Group 1; Fig. 2A). On the other hand, VEGF values were significantly associated between primary tumor and matched liver metastasis (Fig. 2B; $p=0.0083$, $r_s=0.482$) and IL-8 (Fig. 2C, $p=0.034$, $r_s=0.39$).

Correlation in mRNA expression between COX-2 and angiogenic factors in primary colorectal cancer. The mRNA expression of COX-2 significantly correlated with that of VEGF in primary tumors from Group 1 patients (Fig. 3A; $p=0.044$, $r_s=0.37$) and IL-8 (Fig. 3B; $p=0.0004$, $r_s=0.64$). Multivariate analysis revealed that IL-8 mRNA and COX-2 mRNA expression was independently associated (Table III; $p<0.0001$).

Comparison of mRNA levels between patients with stage II colorectal cancer without recurrence and those with colorectal cancer with liver metastasis. The mRNA levels of primary tumors in Group 1 and 2 patients were as follows: COX-2, .61±0.55 and 0.59±0.78; IL-8, 6.17±7.68 and 6.27±13.43

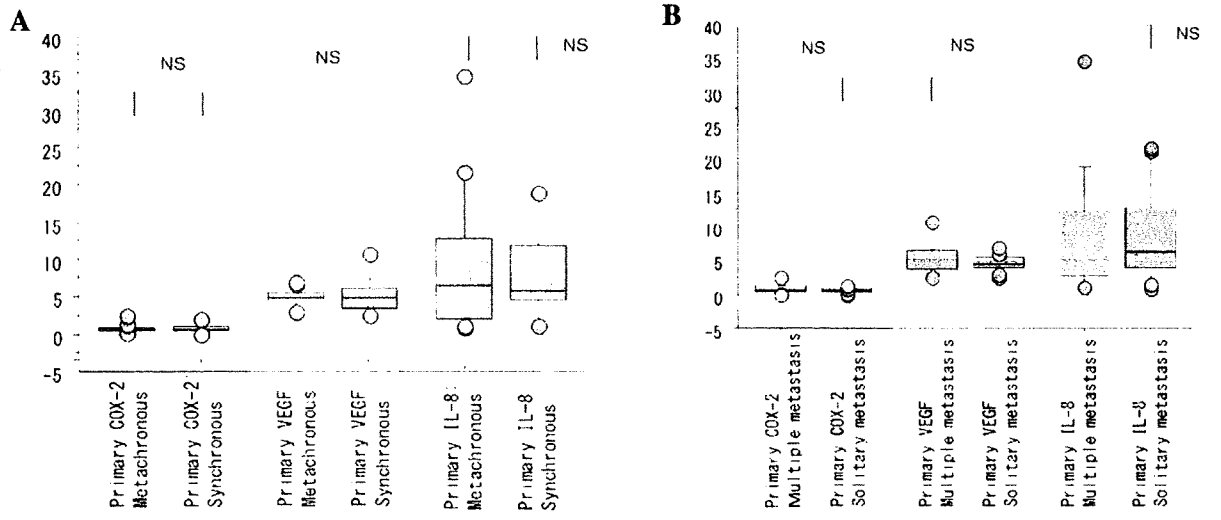


Figure 1. Messenger RNA expression in primary colorectal tumor of patients with liver metastasis according to: (A), timing of metastasis; and (B), number of metastatic tumors.

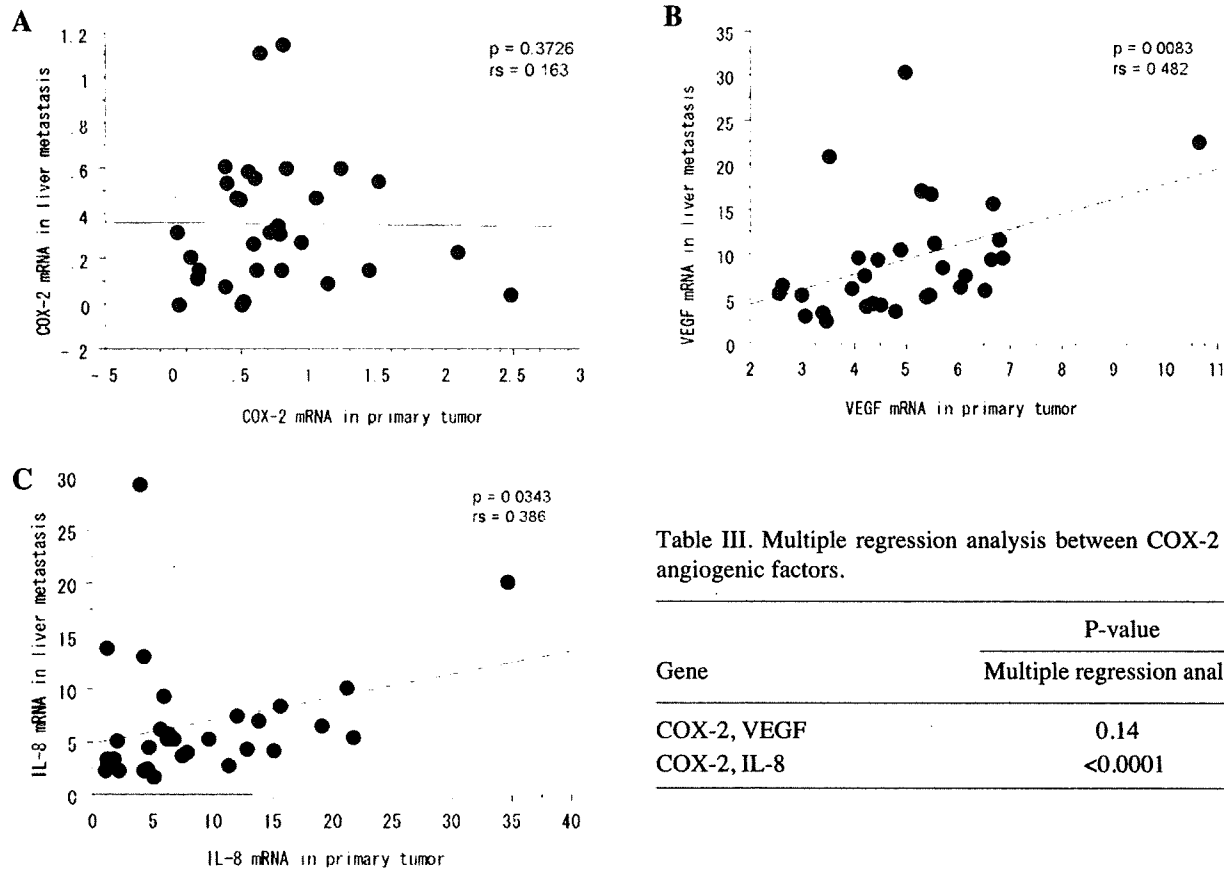


Figure 2. Correlation of messenger RNA expression between primary colorectal cancer and corresponding liver metastasis. (A), COX-2; (B), VEGF; and (C), IL-8.

and VEGF, 4.87±1.64 and 5.50±4.50, respectively. The COX-2 (p=0.55; Fig. 4A), IL-8 (p=0.61; Fig. 4B) and VEGF (p=0.22; Fig. 4C) mRNA levels did not differ between Groups 1 and 2.

Table III. Multiple regression analysis between COX-2 and angiogenic factors.

Gene	P-value Multiple regression analysis
COX-2, VEGF	0.14
COX-2, IL-8	<0.0001

Discussion

The present study demonstrated positive correlations between mRNA levels of IL-8 and VEGF, but not of COX-2 in primary

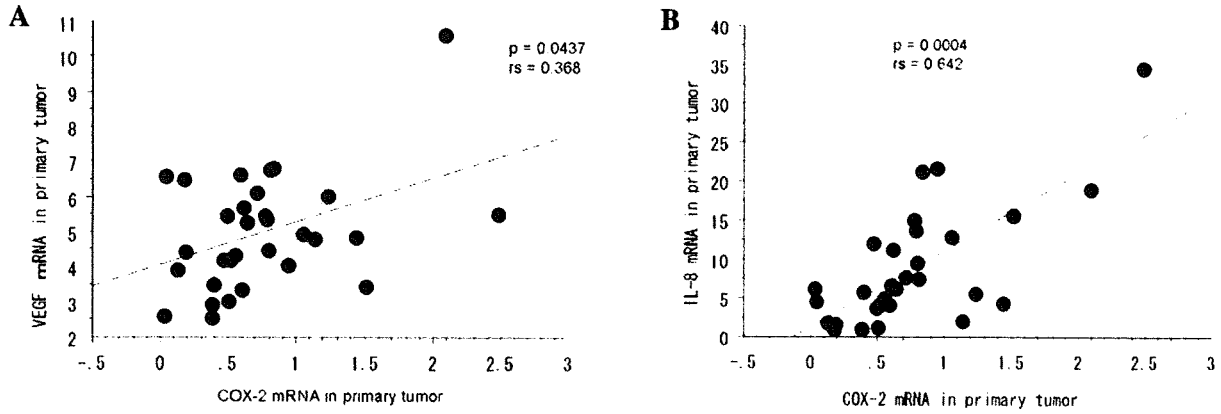


Figure 3. Correlation of messenger RNA expression between COX-2 and: (A), VEGF; or (B), IL-8 in primary tumors.

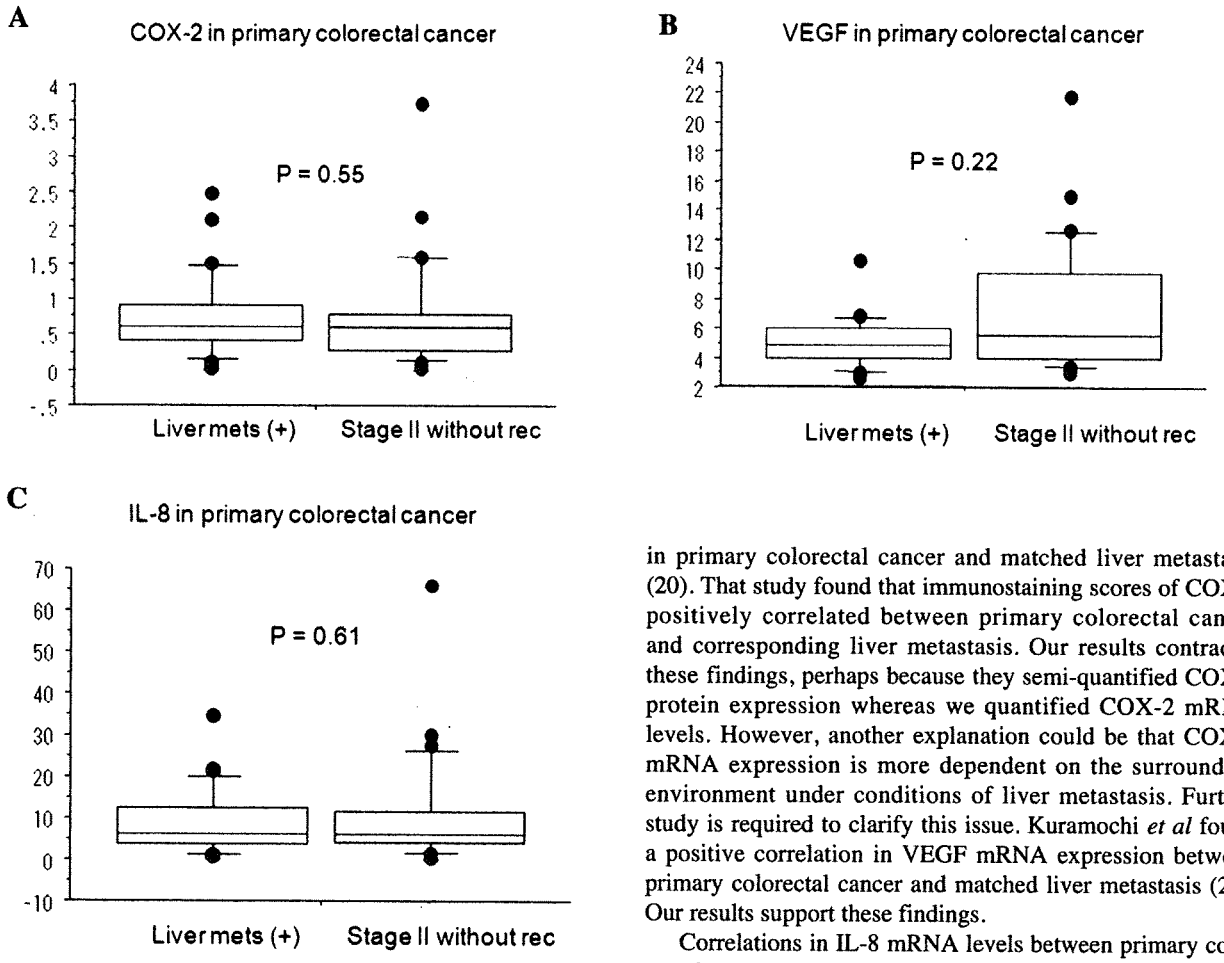


Figure 4. Comparison of messenger RNA expression between primary colorectal cancer with liver metastasis and stage II tumor without recurrence. (A), COX-2; (B), VEGF; and (C), IL-8.

colorectal cancer and corresponding liver metastases. The expression of COX-2 in primary colorectal cancer and liver metastasis has not been examined in detail. Only one immunohistochemical study has compared COX-2 expression in primary colorectal cancer and matched liver metastasis (20). That study found that immunostaining scores of COX-2 positively correlated between primary colorectal cancer and corresponding liver metastasis. Our results contradict these findings, perhaps because they semi-quantified COX-2 protein expression whereas we quantified COX-2 mRNA levels. However, another explanation could be that COX-2 mRNA expression is more dependent on the surrounding environment under conditions of liver metastasis. Further study is required to clarify this issue. Kuramochi *et al* found a positive correlation in VEGF mRNA expression between primary colorectal cancer and matched liver metastasis (21). Our results support these findings.

Correlations in IL-8 mRNA levels between primary colorectal cancer and corresponding liver metastasis have not been reported. Rubie *et al* reported that IL-8 mRNA and protein expression is up-regulated in colorectal cancer compared with adjacent normal tissues (22). Anti-angiogenic therapy for colorectal cancer targeting IL-8 might be developed soon, and the present results should be applicable at that time.

Multiple regression analysis revealed a significant correlation between mRNA levels of IL-8 and of COX-2 in advanced colorectal cancer. To our knowledge, the association

between COX-2 and IL-8 mRNA expression in colorectal cancer has not yet been reported. However, details of interactions between COX-2 and IL-8 were not clarified in the present study. Singh *et al* reported that COX-2 expression led to IL-8 induction in breast cancer cells (23). A similar mechanism might exist in colorectal cancer, because we found a close correlation between the mRNA levels of COX-2 and IL-8. Details of the mechanism between COX-2 and IL-8 in colorectal cancer require further investigation using various strategies.

Since Tsujii *et al* reported that COX-2 regulates angiogenesis in colon cancer cells (24), several studies have shown an association between COX-2 expression and angiogenesis (16,17). However, our univariate analysis found that COX-2 mRNA expression in primary colorectal cancer positively correlated with VEGF mRNA levels, whereas multivariate analysis did not. One reason for this finding might be that several factors other than COX-2 affect VEGF and thus, angiogenesis.

The present study found no differences among COX-2, VEGF, and IL-8 mRNA levels in primary colorectal cancer between patients with synchronous and metachronous liver metastases. The mRNA levels of each factor did not differ between primary tumors from patients with solitary liver or multiple liver metastases, suggesting that these genes are already sufficiently up-regulated by the time liver metastases develop from colorectal cancer. Therefore, the mRNA levels of these genes might not change with further tumor advances.

We found no difference in the IL-8 mRNA levels between TNM stage II and IV primary colorectal cancer. There were no differences in the COX-2 and VEGF mRNA levels between two groups, either. These findings suggest that the IL-8 as well as COX-2 and VEGF mRNA levels in colorectal cancer are already sufficiently up-regulated at stage II. Anti-angiogenic therapy targeting these genes may exert their effect for patients with stage II colorectal cancer as well as for those with stage IV. To maximally exclude bias, we examined samples from consecutive patients with stage II cancer who had not developed recurrence for at least 3 years. Terada *et al* reported that the IL-8 levels were lower in T1, than in T2-4 colorectal cancer (25). Therefore, IL-8 might become up-regulated early. They found higher IL-8 levels in patients with, than without liver metastases. One explanation for the difference in the results between their study and ours might be that they measured IL-8 levels using an ELISA in only 9 patients with liver metastasis. Further large-scale investigations are required to clarify this issue.

In conclusion, the present study found no association between mRNA expression of angiogenic factors and liver metastasis. The mRNA expression of these angiogenic factors in colorectal cancer might already be sufficiently up-regulated before hematogenous metastasis. The angiogenic activity of COX-2 might be exerted more through the IL-8 than the VEGF pathway. The mRNA levels of VEGF and IL-8 in liver metastasis can be predicted from those in primary colorectal cancer. These findings will be useful when considering anti-angiogenic therapy for patients with colorectal cancer, although further studies are required to validate these preliminary data.

Acknowledgments

We thank Yoko Takagi for her excellent technical assistance. Part of this study was presented at the annual AACR meeting, San Diego, April 12-16, 2008.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ: Cancer statistics, 2008. CA Cancer J Clin 58: 71-96, 2008.
2. Muto T, Kotake K and Koyama Y: Colorectal cancer statistics in Japan: data from JSCCR registration, 1974-1993. Int J Clin Oncol 6: 171-176, 2001.
3. Kobayashi H, Mochizuki H, Sugihara K, Morita T, Kotake K, Teramoto T, Kameoka S, Saito Y, Takahashi K, Hase K, Oya M, Maeda K, Hirai T, Kameyama M, Shirouzu K and Muto T: Characteristics of recurrence and surveillance tools after curative resection for colorectal cancer: a multicenter study. Surgery 141: 67-75, 2007.
4. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R and Kabbinavar F: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350: 2335-2342, 2004.
5. Folkman J: Endothelial cells and angiogenic growth factors in cancer growth and metastasis. Introduction. Cancer Metastasis Rev 9: 171-174, 1990.
6. Baggiolini M, Dewald B and Moser B: Human chemokines: an update. Annu Rev Immunol 15: 675-705, 1997.
7. Matsushima K and Oppenheim JJ: Interleukin-8 and MCAF: novel inflammatory cytokines inducible by IL 1 and TNF. Cytokine 1: 2-13, 1989.
8. Oppenheim JJ, Zachariae CO, Mukaida N and Matsushima K: Properties of the novel proinflammatory supergene 'intercrine' cytokine family. Annu Rev Immunol 9: 617-648, 1991.
9. Rossi D and Zlotnik A: The biology of chemokines and their receptors. Annu Rev Immunol 18: 217-242, 2000.
10. Koch AE, Polverini PJ, Kunkel SL, Harlow LA, DiPietro LA, Elner VM, Elner SG and Strieter RM: Interleukin-8 as a macrophage-derived mediator of angiogenesis. Science 258: 1798-1801, 1992.
11. Brew R, Southern SA, Flanagan BF, McDicken IW and Christmas SE: Detection of interleukin-8 mRNA and protein in human colorectal carcinoma cells. Eur J Cancer 32A: 2142-2147, 1996.
12. Fox SH, Whalen GF, Sanders MM, Burleson JA, Jennings K, Kurtzman S and Kreutzer D: Angiogenesis in normal tissue adjacent to colon cancer. J Surg Oncol 69: 230-234, 1998.
13. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS and Dvorak HF: Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219: 983-985, 1983.
14. Jones DA, Carlton DP, McIntyre TM, Zimmerman GA and Prescott S: Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines. J Biol Chem 268: 9049-9054, 1993.
15. Williams CS and DuBois RN: Prostaglandin endoperoxide synthase: why two isoforms? Am J Physiol 270: G393-G400, 1996.
16. Kobayashi H, Gonda T, Uetake H, Higuchi T, Enomoto M and Sugihara K: JTE-522, a selective COX-2 inhibitor, interferes with the growth of lung metastases from colorectal cancer in rats due to inhibition of neovascularization: a vascular cast model study. Int J Cancer 112: 920-926, 2004.
17. Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM, Edwards DA, Flickinger AG, Moore RJ and Seibert K: Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 60: 1306-1311, 2000.
18. Lord RV, Salonga D, Danenberg KD, Peters JH, DeMeester TR, Park JM, Johansson J, Skinner KA, Chandrasoma P, DeMeester SR, Bremner CG, Tsai PI and Danenberg PV: Telomerase reverse transcriptase expression is increased early in the Barrett's metaplasia, dysplasia, adenocarcinoma sequence. J Gastrointest Surg 4: 135-142, 2000.
19. Gibson UE, Heid CA and Williams PM: A novel method for real-time quantitative RT-PCR. Genome Res 6: 995-1001, 1996.

20. Nakamoto RH, Uetake H, Iida S, Kolev YV, Soumaoro LT, Takagi Y, Yasuno M and Sugihara K: Correlations between cyclooxygenase-2 expression and angiogenic factors in primary tumors and liver metastases in colorectal cancer. Jpn J Clin Oncol 37: 679-685, 2007.
21. Kuramochi H, Hayashi K, Uchida K, Miyakura S, Shimizu D, Vallbohmer D, Park S, Danenberg KD, Takasaki K and Danenberg PV: Vascular endothelial growth factor messenger RNA expression level is preserved in liver metastases compared with corresponding primary colorectal cancer. Clin Cancer Res 12: 29-33, 2006.
22. Rubie C, Frick VO, Pfeil S, Wagner M, Kollmar O, Kopp B, Graber S, Rau BM and Schilling MK: Correlation of IL-8 with induction, progression and metastatic potential of colorectal cancer. World J Gastroenterol 13: 4996-5002, 2007.
23. Singh B, Berry JA, Vincent LE and Lucci A: Involvement of IL-8 in COX-2-mediated bone metastases from breast cancer. J Surg Res 134: 44-51, 2006.
24. Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M and DuBois RN: Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 93: 705-716, 1998.
25. Terada H, Urano T and Konno H: Association of interleukin-8 and plasminogen activator system in the progression of colorectal cancer. Eur Surg Res 37: 166-172, 2005.

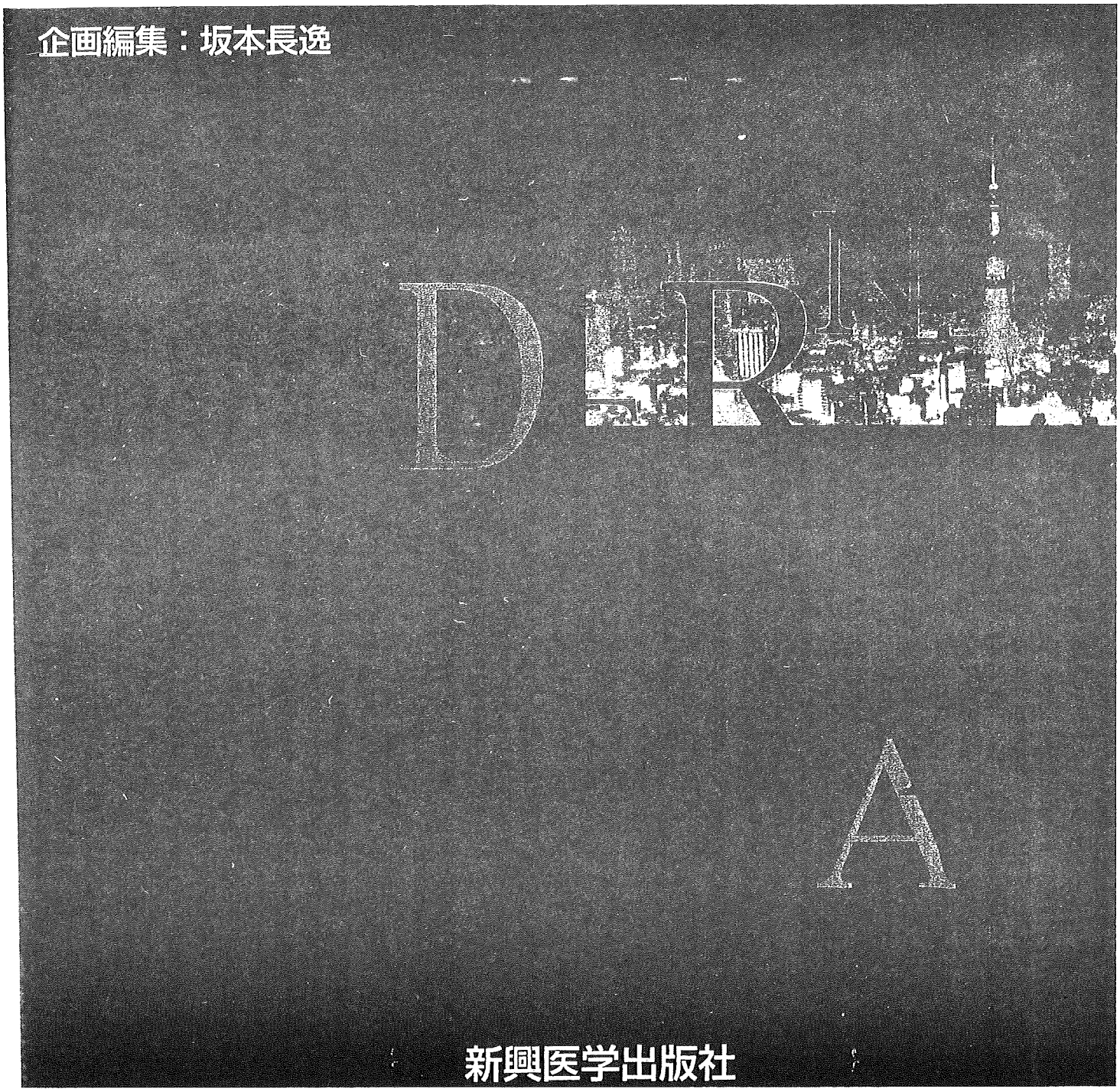
第29巻第7号　平成21年7月1日発行（毎月1日発行）　昭和57年4月22日　第3種郵便物認可　ISSN 0913-7963

モダンフィジシャン
内科系総合雑誌
VOL29
NO. 7

未来の消化器医療を採る

- 分子生物学と未来の消化器医療
- 外科と未来の消化器医療　■内科と未来の消化器医療
- 内視鏡医療の未来　■放射線診断学と消化管診断学の未来
- 病理診断学と未来の消化器医療
- 特別寄稿『日本の医学・医療の未来を語る』

企画編集：坂本長逸



新興医学出版社

今月のアプローチ

特集：未来の消化器医療を探る

アジュバント/ネオアジュバント化学療法の進歩と未来

石川　敏昭*　植竹　宏之*　杉原　健一**
いしかわ　としあき　うえたけ　ひろゆき　すぎはら　けんいち

● 補助化学療法の有用性は無作為比較試験における予後改善の有無により検証される．近年，有効な抗癌剤の開発とそれを用いた大規模無作為化比較試験の成果により補助化学療法の有用性の実証と標準治療の確立が進んでいる．
● わが国と欧米では手術術式や治療成績に違いがある．欧米の臨床試験の結果を合理的に導入しつつ，わが国の癌治療に適した標準治療の確立を進めることが重要である．
● 現在の補助化学療法では手術単独で治癒する患者も対象に含まれている．治療効果の向上だけでなく，副作用や経済的負担の軽減も重要である．適切な患者に有効かつ副作用の少ない薬剤を投与する個別化治療の確立が求められている．
● 個別化治療には指標となるバイオマーカーが必要である．実用性の高いバイオマーカーの同定を試みるトランスレーショナル研究が進められており，今後は，同定したバイオマーカーを用いた個別化治療の有用性を検証する無作為比較試験が重要になる．

Key Words　補助療法，標準治療，個別化治療，バイオマーカー，トランスレーショナル研究

消化器癌の治療では，手術治療が唯一，治癒の期待できる治療方法である．しかし，進行例では治癒切除後であっても再発する場合があり，再発例の予後は不良である．補助療法の目的は治癒切除後の再発を抑制し，予後を向上させることである．今までも補助化学療法による予後の改善が期待されてきたが，科学的根拠は十分ではなかった．近年，有効な抗癌剤の開発と大規模無作為化比較試験の成果により，補助化学療法の有用性の実証と標準治療の確立が進んでいる．本稿では，消化器癌に対する補助化学療法の進歩と標準化の現状およびその将来として個別化治療の展望を概説する（図1）

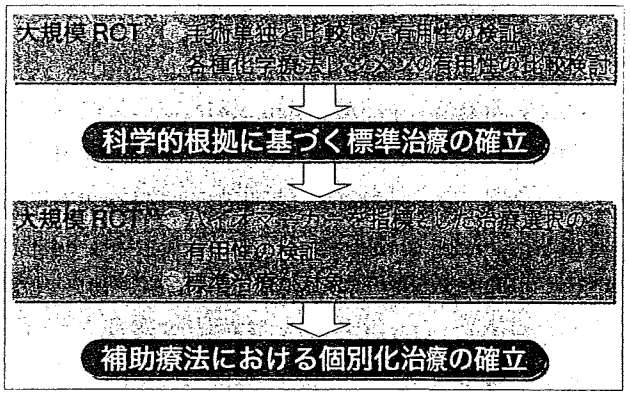


図1　補助化学療法の進歩と将来

□ 補助化学療法とは

一般に腫瘍量が少ないほど抗癌剤耐性細胞が少なく，抗腫瘍効果も高いと考えられており，治癒切除術後に遺残している可能性がある癌細胞の根絶が補助化学療法に期待されてきた．

1．ネオアジュバント/アジュバント化学療法

ネオアジュバント化学療法は，治癒手術が計画されている症例に術前に行う補助療法を意味し，切除不能進行癌に対する化学療法が奏効して結果的に根治切除が可能になった場合とは区別される．術前補助化学療法の利点は，病期の改善による機能温存手術の可能性があることや切除検体の組織学的検索により薬剤感受性の判定が可能であることがあげられる．不利な点としては，無効であった場合病期が進行し，手術の根治性が損なわれる危険性や手術合併症が増加する可能性がある．一

*東京医科歯科大学大学院　応用腫瘍学　　**同　腫瘍外科学

0913-7963/09/¥800/論文/JCOPY